Original Article

Morphometric Characteristics and Species Identification of Bed Bugs (Hemiptera: Cimicidae) in Eastern Iran

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Abstract

Background: The common bed bugs, *Cimex lectularius*, and tropical bed bugs, *Cimex hemipterus* are the primary species of public health importance in the family Cimicidae. This study aimed to determine the morphometric criteria and prevalent species of bed bugs in eastern Iran.

Methods: Bed bugs were collected from March 2021 to June 2022 from infested residential buildings and apartments in eastern Iran, including North Khorasan, Khorasan Razavi, and Sistan and Baluchistan Provinces. The morphological and morphometrical characteristics were used to identify collected bed bugs at inter- and intra-specific levels.

Results: A total of 34 isolates comprising 127 adult bed bugs were collected from Bojnord, Mashhad, Neishabur, Taibad, Sabzevar, Kashmer, Zahedan, Saravan, Rask, Pishin and Chabahar. Of these, 33 isolates (n=124) were found to be tropical bed bugs, *C. hemipterus*, and one population (n=3) was identified as *C. lectularius*. The index pronotal width/length ratio was calculated from 2.72 to 2.94 and 1.98 to 2.47 for *C. lectularius* and *C. hemipterus*, respectively. The length/width ratio of the hind femur was 3.365 in *C. hemipterus* and 4.267 in *C. lectularius*. The ratio of length/width of the third femur (F3 l/w) between populations of *C. hemipterus* was different, and this difference was statistically significant ($P< 0.05$).

Conclusion: The results of this study indicated that *C. hemipterus* was the dominant bed bug species in the east of Iran and provided more morphometric criteria of *C. hemipterus* for researchers to identify the species and determine the intraspecific variations in the present and future.

Keywords: Bed bugs; Biology; Humans; Public health; Iran

Introduction

The common bed bug, *Cimex lectularius*, with a global distribution, and the tropical bed bug, *Cimex hemipterus* distributed chiefly in tropical countries, are the most important species of the family Cimicidae from the public health perspective. Infestation of human dwellings with this parasite can adversely affect human health and welfare through blood-sucking behavior during the night. However, the transmission of any pathogenic agents to humans through biting of bed bugs in natural conditions has not been reported (1). Its global extent, developing insecticide resistance, and difficulty in treatment have made this insect a critical problem in urban pest management. The distribution pattern of bed bugs is changing around the world. The presence of *C. hemipterus* in Russia and France was reported for the first time by Gapon (2) and Bérenger and Pluot-Sigwalt (3), respectively. In the past ten years, *C. lectularius* has given way to *C. hemipterus* in the cities of Iran that were studied, and *C. hemipterus* has become more dominant than before (4). Demographic dynamics and changes

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can occur for various reasons. However, the expansion of tourism, the development of domestic and international travel, and the phenomenon of global warming may be part of these reasons (5).

Leptocimex boueti is a less common species that can feed on human blood in limited areas, especially where people live near bat habitats or visit these places (6). Other genera and species of the family Cimicidae are less important than the abovementioned species from the point of view of human health. Accurate diagnosis of bed bug species based on morphological interspecific differences and attention to intraspecific morphological and morphometrical variations, along with tracking these variations, can be helpful for more accurate species identification and understanding of the speciation process.

Morphometrics is one of the best quantitative approaches to taxonomy, phenotypic, and genotypic evolution (7). This branch of biology has contributed to a better understanding of valuable phenomena such as phylogenetic and ecomorphological relationships, life histories, animal behavior, community structures, and ecological processes (8). Inter/intraspecific differences can be detectable with repeatable results by this approach if experts follow the same standards (9). Furthermore, morphometric findings are widely transferable and can become a reliable data source for alpha classification (10).

Following the initial report of the presence of *C. hemipterus* in Iran (4), subsequent researches have also confirmed the existence of tropical bed bugs in the western and central regions of the country (11, 12). Bahrami et al. (13) studied the phylogenetic and ultrastructure characterization of bed bugs in southwestern Iran and showed that *C. hemipterus* was the predominant species in that region. However, there is currently no available data regarding the dominant bed bug species in eastern Iran, nor any information on their morphometric characteristics. This study aimed to determine the morphometric criteria and prevalent species of bed bugs in eastern Iran.

Materials and Methods

This study was conducted from March 2021 to June 2022. The study area included four provinces North Khorasan, Khorasan Razavi, South Khorasan, and Sistan and Baluchistan, located in the east of Iran (Fig. 1). This area was selected due to a need for more concise scientific reports regarding bed bug infestations and related species in the cities of these provinces. Khorasan Razavi is the most crowded province in sampled areas with a population of 6434501, followed by Sistan and Baluchistan with 2775014, North Khorasan with 850,000, and South Khorasan with 768898 population. The study area is situated between latitudes 25°03'34.1" N- 38°16'48.5" N and longitudes 55°23'11.3" E- 63°20'00.9" E.

Adult bed bugs were collected from infested residential buildings and apartments reported by pest control companies in the study areas. Bed bugs were hand-collected using soft forceps transferred to plastic containers with tight lids and preserved in ethanol 96%. The specimens were taken to the parasitology laboratory, where each bed bug was placed between two slides and fixed by a pair of hair clips, then examined under a stereo microscope. The standard morphological keys were used to identify bed bugs based on their morphological features (14).

The methodology described by Balvin et al. (15) was used to assess the variation of different body parts of collected specimens. Morphometric characteristics were photographed by a digital camera (Vega 3146200 - Labomed, USA) under a stereomicroscope and measured using Image J software (version 1.52). Forty-six indices, including total body length (tl), width (tw), length of antennal segments (al1-al4), head length (hl) and width (hw), length (ed) and width (ew) of eyes, the intraocular space dorsally (is) and ventral (iv) surfaces, length of the longest hair between eyes and antennae (se), the width of clypeus (cw), length of hair on clypeus (sc), length of proboscis segments (rl1- rl3),

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length (pl) and width (pw) of pronotum, length of the middle part of pronotum (pm), depth of pronotal concavity (pc), length of setae on the pronotum (sp), length (sl) and width (sw) of scutellum, length (lh) and width (wh) of hemelytra, length of setae on hemelytra (sh), length (fl1-fl3) and the width (fw1-fw3) of the femurs and the length (tl1-tl3) and width (tw1-tw3) of the tibias, total antenna length (alt) and total rostellum length (rlt) were measured and recorded (Fig. 2). Some important ratios were also calculated, such as the ratio of pronotum width/length (pw/l) and hind femur length/width (f3l/w). The most extended distances were considered for the records; to measure the length of the setae, the longest was considered, and in measuring the width of the tibia, measurement was conducted almost in the middle of the tibia.

The data of indices obtained from males and females, as well as width/length of pronotum (pw/l), length/width of head, length/width of 3rd femur (f3 l/w), length of seta of pronotum (sp), head width/eye width (hw/ew), scutellum width/head width (sw/hw) and hemelytra length/hemelytra width of *C. hemipterus* specimens, were selected and submitted to SPSS software (Version 16). All data were assessed for normality by the Kolmogorov-Smirnov test. The data sets with non-normal distribution were compared using Kruskal-Wallis and Mann-Whitney tests. P values< 0.05 were considered statistically significant.

Results

A total of 34 isolates comprising 127 adult bed bugs, including 61 females and 66 males, were collected from Bojnord, Mashhad, Neishabur, Taibad, Sabzevar, Kashmer, Zahedan, Saravan, Rask, Pishin and Chabahar. However, we could not find any bed bug infestation in the cities of South Khorasan Province. All collected specimens were identified based on morphometric characteristics to species level. Of these, 33 isolates (n=124) were found to be the tropical bed bugs, *C. hemipterus*, and one population (n=3) was identified as the common bed bug, *C. lectularius* (from an archived collection in Mashhad, Iran, collected in 2007). The index pronotal width/length ratio was calculated from 2.72 to 2.94 and 1.98 to 2.47 for *C. lectularius* and *C. hemipterus*, respectively (Fig. 3).

In this study, the body length and width of *C. hemipterus* were 4.43–7.7 mm and 2.27–3.32 mm, respectively. The body length of *C. lectularius* was 5.3–5.4, and its width was measured at 2.7–3.4 mm. The mean size of the body width of females was significantly higher than that of males, whereas their difference in body length was insignificant. Detailed data of other measured indices and their significance between males and females can be found in Table 1.

The ratio of length/width of the third femur (F3 l/w) between populations of *C. hemipterus* was different, and this difference was statistically significant ($P< 0.05$).

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Table 1. Measured morphometric characteristics of male and female *C. hemipterus* collected from east of Iran (n=124)

Index	Female			Male			P-Value
	n	Mean (mm)	SD	n	Mean (mm)	SD	
Total body length (tl)	53	5.538	0.726	62	5.319	0.550	0.069
Total body width (tw)	53	2.955	0.163	62	2.687	0.158	0.000
1st antennal segment (al1)	53	0.190	0.016	62	0.188	0.015	0.365
2nd antennal segment (al2)	54	0.648	0.038	61	0.620	0.033	0.000
3rd antennal segment (al3)	33	0.603	0.043	38	0.583	0.041	0.042
4th antennal segment (al4)	27	0.449	0.036	37	0.435	0.032	0.166
Total length of antenna (alt)	26	1.877	0.109	35	1.821	0.089	0.035
Head width (hw)	58	0.995	0.034	65	0.955	0.037	0.000
Head length (hl)	57	0.813	0.051	64	0.788	0.052	0.014
Eye width (ew)	58	0.126	0.013	65	0.121	0.012	0.039
Eye length (ed)	58	0.237	0.019	65	0.227	0.018	0.002
Intraocular space dorsally (is)	58	0.742	0.031	65	0.714	0.037	0.000
Intraocular space ventral (iv)	55	0.767	0.048	62	0.730	0.041	0.000
Seta between eyes and antennae (se)	58	0.069	0.010	65	0.071	0.008	0.273
Width of clypeus (cw)	58	0.367	0.015	65	0.357	0.016	0.001
Length of seta on clypeus (sc)	56	0.072	0.008	65	0.072	0.007	0.966
1st segment of rostellum (rl1)	49	0.444	0.040	51	0.430	0.038	0.088
2nd segment of rostellum (rl2)	52	0.285	0.030	59	0.269	0.028	0.007
3rd segment of rostellum (rl3)	53	0.329	0.026	60	0.320	0.024	0.028
Total length of rostellum (rlt)	48	1.060	0.049	50	1.022	0.048	0.000
Pronotum width (pw)	59	1.408	0.066	65	1.356	0.061	0.000
Pronotum length (pl)	58	0.821	0.044	64	0.796	0.048	0.012
Pronotum length medially (pm)	59	0.629	0.030	64	0.611	0.039	0.004
Width to length of pronotum medially (pwl)	59	2.240	0.086	64	2.227	0.088	0.647
Depth of pronotal concavity (pc)	58	0.185	0.027	64	0.178	0.024	0.109
Length of setae on the pronotum (sp)	58	0.099	0.021	64	0.098	0.022	0.790
Scutellum width (sw)	58	0.879	0.050	65	0.837	0.048	0.000
Scutellum length (sl)	58	0.395	0.035	65	0.382	0.033	0.028
Hemlytra length (lh)	58	0.608	0.042	64	0.578	0.045	0.000
Hemlytra width (wh)	58	0.931	0.059	64	0.880	0.065	0.000
Setae on hemlytra (sh)	58	0.104	0.029	64	0.109	0.028	0.238
Length of 1st femur (fl1)	54	1.121	0.048	62	1.100	0.066	0.040
Width of 1st femur (fw1)	54	0.369	0.022	62	0.353	0.020	0.000
Length of 1st tibia (tl1)	51	1.165	0.058	61	1.117	0.062	0.000
Width of 1st tibia (tw1)	53	0.114	0.006	61	0.110	0.007	0.000
Length of 2nd femur (fl2)	54	1.175	0.055	63	1.146	0.074	0.015
Width of 2nd femur (fw2)	54	0.382	0.021	63	0.362	0.021	0.000
Length of 2nd tibia (tl2)	50	1.256	0.077	63	1.200	0.066	0.000
Width of 2nd tibia (tw2)	51	0.116	0.009	63	0.110	0.007	0.000
Length of 3rd femur (fl3)	55	1.339	0.060	64	1.299	0.076	0.004
Width of 3rd femur (fw3)	55	0.405	0.023	64	0.379	0.024	0.000
Length to width of 3rd femur (f3lw)	55	3.308	0.155	64	3.428	0.162	0.000
Length of 3rd tibia (tl3)	51	1.731	0.114	62	1.638	0.101	0.000
Width of 3rd tibia (tw3)	51	0.122	0.009	62	0.113	0.007	0.000
Length to width of the head (hl/hw)	57	0.816	0.040	64	0.826	0.042	0.063
Head width to eye width (hw/ew)	58	7.992	0.847	65	7.941	0.738	0.859

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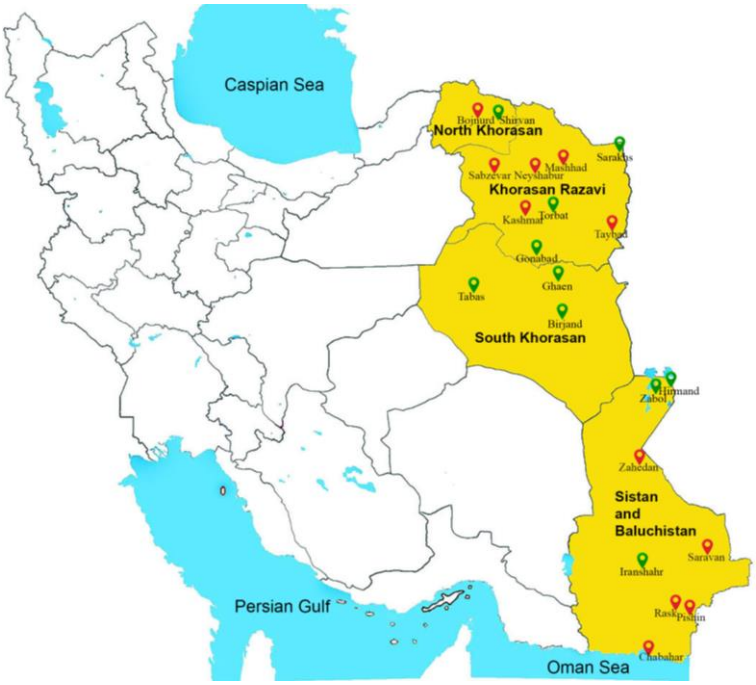

Fig. 1. The map shows I. R. Iran and the yellow-colored study area, with red and green location points indicating respectively the cities with positive and negative bed bug infestation, 2021–2022

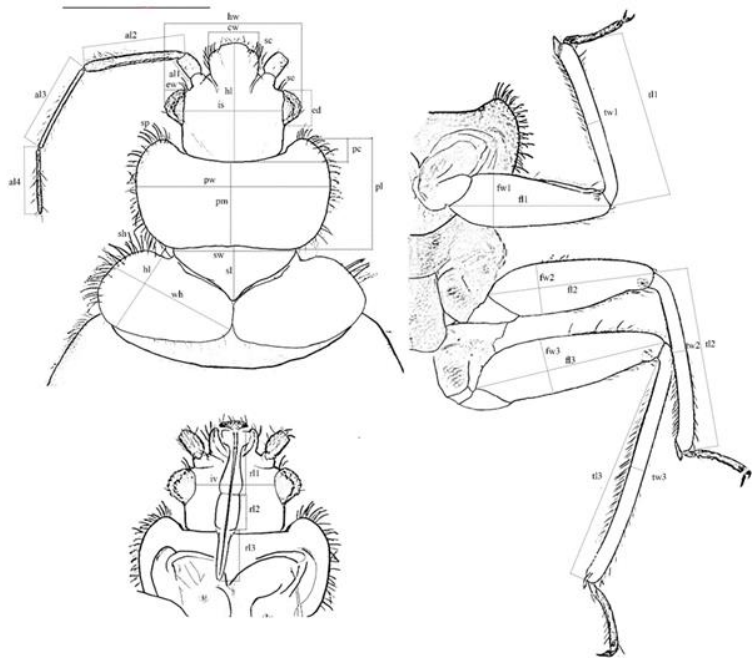

Fig. 2. Measured indices used in the morphological analysis. Length of antennal segments (al1-al4), head length (hl) and width (hw), length (ed) and width (ew) of eyes, the intraocular space dorsally (is) and ventral (iv) surfaces, length of the most extended hair between eyes and antennae (se), the width of clypeus (cw), length of hair on clypeus (sc), length of probosci's segments (rl1-rl3), length (pl) and width (pw) of pronotum, length of the middle part of pronotum (pm), depth of pronotal concavity (pc), length of setae on the pronotum (sp), length (sl) and width (sw) of scutellum, length (lh) and width (wh) of hemelytra, length of setae on hemlytra (sh), length (fl1-fl3) and the width (fw1-fw3) of the femurs and the length (tl1-tl3) and width (tw1-tw3) of the tibias (The figure is original and abbreviations adapted from Balvin et al. (12))

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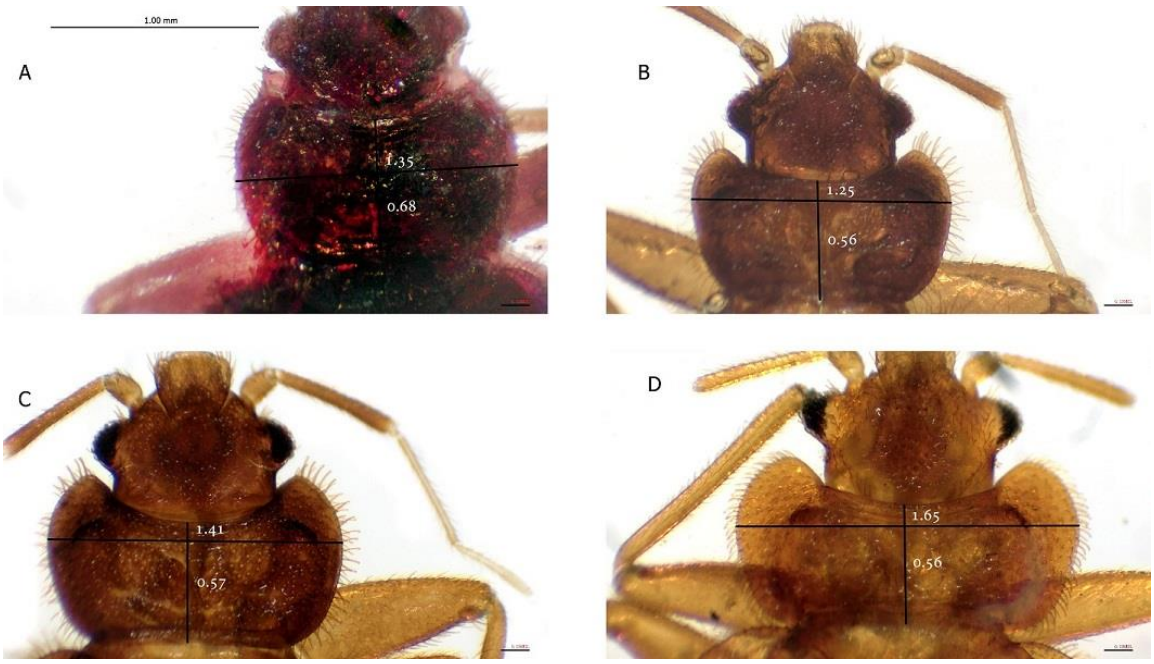

Fig. 3. Pronotum and head of adult *Cimex hemipterus* (A, B, C) from three distinct populations and *Cimex lectularius* (D). The horizontal line on the pronotum has demonstrated intra/interspecific variation in the width size of the pronotum

Discussion

Since bed bugs are of medical importance, correct species identification is essential in managing their infestations. This study measured and recorded 46 morphometric indices of collected bed bugs from the east of Iran. Some of these indices were measured and presented in this study for the first time. Although the previous studies have tried to present some morphometric features, the present investigation attempted to provide the most. These data can improve our knowledge of morphological and morphometrical variations in different geographical regions. In addition, these data allow us to track the changes in these indices and speciation over time.

The morphometric studies showed that all bed bugs collected from the east of Iran belonged to the species *C. hemipterus*, and we found *C. lectularius* specimens in an archived collection from Mashhad. *Cimex lectularius* in Iran was reported from northern Iran for the first time by Lindberg in 1938 (16), and there was a record of this species in Tehran in 1950 (17). From then until 2019, *C. lectularius* was reported from different regions of Iran. In 2019, bed bug samples collected from Tehran and Khorramabad were identified as *C. hemipterus* based on morphological characteristics and confirmed by molecular assay (4). Since then, this species has become the dominant and prevalent species of the genus *Cimex* reported from Iran. Bahrami et al. (13) identified collected bed bugs from Ahvaz in southwestern Iran as *C. hemipterus* based on morphological characters and molecular phylogeny. *Cimex hemipterus* was reported from northwestern and the west of Iran based on unmentioned morphological criteria and molecular studies (11). Some reports from other regions of the country indicate the presence of *C. hemipterus* (12, 18). *Cimex lectularius* was reported from Mashhad in 2019 (19), and in the present study, we could not find this species in the study area, including Mashhad city. It seems that *C. hemipterus* has become the dominant bed bug species in the study area and areas that have even previ-

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ously been home to *C. lectularius*. This phenomenon can be attributed to climatic conditions and tourism development based on more trips to neighboring countries and Southeast Asia, where this species is more common.

The identification of bed bugs at the species level is usually based on morphological characteristics such as pronotum, hind tibial, paragenital sinus, and paramer (12). The pronotum width-to-length ratio is the most influential factor for differentiating *C. hemipterus* and *C. lectularius*. Based on our findings *C. hemipterus* pronotum was less than 2.5 times as wide as long at the middle and more than 2.5 times for *C. lectularius*. Usinger (14) described the pronotum of *C. hemipterus* as slightly more than twice as wide as long but 2.5 times as wide as long in *C. lectularius*. The ratio of width to length of pronotum was reported as 2.1 and 2.1–2.37 in *C. hemipterus* collected from Malaysia and Russia, respectively (2, 20). This data showed intraspecific variations in pronotum width-to-length ratio among *C. hemipterus* populations and specimens collected from different locations. Although bed bugs can be identified based on other morphometric criteria or molecular characteristics, calculating the index of width to length of the pronotum is inexpensive, fast, and highly reliable for species identification.

We observed morphometrical variation between the two sexes, and females were the larger sex. According to Adams and Funk (21), sexual body size dimorphism is commonly observed in invertebrate taxa. This size difference is related to abdomen size as an adaptive advantage for greater fecundity in females (22). Blood intake of adult females of *C. hemipterus* was reported to be 1.4 to 2.0 times more than adult males and this variation in the blood meal size can affect the body size of individuals (23). Although the body length is traditionally used by some researchers (13, 24, 25), high intraspecific variations were observed in the body length of collected populations. Therefore, it cannot serve as a reliable index for species identification. Body length is highly dependent on the size of the abdomen, and the latter is affected by gender and the amount of blood ingested. The correlation between collection sites and body size was not examined in the present study. However, some researchers concluded that a variation in size between populations can primarily be related to environmental conditions (26).

The fourth segment of the antenna was shorter, and the ratio of intraocular space to eye width was higher compared to *C. hemipterus* specimens studied by Usinger (14). Although these differences can reflect intraspecific variations in populations studied in two different geographic locations, they may be related to the conditions under which samples were preserved and fixed for morphometric studies. We preserved and fixed collected bed bugs in ethanol 96% while dried samples were used for morphometric analysis by Usinger (14). Considering that many samples were measured in both studies, the idea of intraspecies variation is more likely.

The length/width ratio of the hind femur was one of the important indices calculated in the current study. The length/width ratio of the hind femur in *C. hemipterus* from India was measured at 3.460 and 3.202 in two studied populations (27). Usinger (14) reported this value as 3.23 for *C. hemipterus* and 3.33 for *C. lectularius*. In the present study, this ratio was 3.365 in *C. hemipterus*, close to those reported in previous studies. It was 4.267 in *C. lectularius*, almost one unit more than the value reported by Usinger (14). This ratio was calculated at 2.71 in bat bugs by Usinger (14), which showed that the hind femur in *C. adjunctus* is wider compared to both bed bug species.

Conclusion

The results of this study indicated that the tropical bed bug, *C. hemipterus* was the dominant bed bug species in the east of Iran. Despite the predominance of *C. lectularius* in the

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recent past, this species was not found in the areas we sampled. The current study also provided more morphometric criteria for researchers to identify the two species and to determine the intraspecific variations in each species.

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Ethical considerations

Ethical approval is not applicable to this study.

Conflict of interest statement

The authors declare there is no conflict of interests.

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